Studying renal vascular changes in progressive kidney diseases by microCT

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Aims
Chronic kidney disease (CKD) represents a major health problem worldwide. It has been estimated that approximately five hundred million individuals in the world are affected by progressive renal diseases, and the costs of renal replacement therapy are continuously increasing. Therefore, it is crucial to identify the mechanisms responsible for CKD progression and to use effective pharmacological treatments able to promote remission or even regression of CKD. Experimental and clinical research has characterized the common features of the evolution of proteinuric progressive kidney diseases. Alterations in glomerular membrane permeability to plasma proteins initiate a cascade of events that lead to proteinuria and tubular protein overload. This condition induces tubular cell changes that are responsible for interstitial damage, tubulo-interstitial fibrosis and glomerulosclerosis. These events progressively lead to complete loss of renal function. Changes in kidney tissue structure during the course of the diseases have been extensively characterized at microscopical level but there is little information about changes in entire kidney vasculature, from renal artery to arcuate arteries and down to glomerular arterioles and capillaries¹. The aim of the study we presented is to investigate renal vascular changes that characterize kidney disease progression in an experimental model of progressive nephropathy by micro-CT investigation and image processing.

Method
We used Munich-Wistar rats by Fromter (MWF) as a model of progressive proteinuric nephropathy. The reason to select these animals relies on the fact that males of this strain develop spontaneous proteinuria and glomerulosclerosis with age. This feature represents an ideal condition to study the mechanism(s) underling renal disease progression avoiding the use of surgery techniques or renal toxins to model the glomerular damage.

Four groups of male rats (4 animals per group) were studied:
Group 1 – normal rats (Wistar) at 25 weeks of age.
Group 2 – MWF rats at 25 weeks of age.
Group 3 – MWF rats at 50 weeks of age.
Group 4 – MWF rats at 60 weeks of age.

Urinary excretion of proteins was measured in 24-hour urine samples by a Cobas Mira autoanalyzer (Roche Diagnostic Systems) at the end of the study.

Before sacrifice, abdominal aorta of the anesthetized animal was cannulated below the renal arteries, the left kidney was perfused with buffered saline containing Heparin (25 U/ml) to remove blood and a radiopaque silicone polymer containing lead chromate (Microfil MV 122; Flow Tech, Carver, MA) was infused through the aortic cannula. When the renal vasculature was filled with the polymer and the kidney had a uniform staining, renal arteries and veins were closed. After 90 minutes the kidney was removed and stored in 4% formaldehyde buffered with 0.1 M phosphate (pH 7.2) to complete fixation². Samples were imaged after three days by Skyscan 1076 system (image pixel size 9 µm, filter Al 0.5, source voltage 47 Kv, source current 200 mA, exposure time 1800 ms, rotation step 0.4°).
Projection images were then reconstructed with NRecon software and analyzed with CTAn. First, we removed part of renal arteries/veins or Microfil traces outside kidney using the ROI tool. Then, we applied two thresholds: threshold=25 to select only kidney; threshold=130 to select vascular volume (i.e. the volume occupied by Microfil). Finally, we computed the kidney volume and the vascular volume for each group using the volume analysis tool.

Results

As shown in Figure 1, MWF rats at 25 weeks had urinary protein excretion higher than aged-matched control Wistar rats. Proteinuria progressively increased with age from 25 to 60 weeks in MWF strain.

![Figure 1: Urinary protein excretion](image)

Using CTVox we performed the volume rendering of the kidney vasculature. In Figure 2, we showed a representative volume rendering for each group. Older MWF rats at 50 and 60 weeks showed simplification of the vascular network, while no differences were observed between Wistar and MWF at 25 weeks.
Quantification of kidney and vascular volumes in Wistar and MWF rats of different age are reported in Table 1.

Table 1: Comparison of kidney volumes and vascular volumes

<table>
<thead>
<tr>
<th></th>
<th>Kidney Volume (KV) [mm$^3$]</th>
<th>Vascular Volume (VV) [mm$^3$]</th>
<th>VV/KV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WISTAR 25 weeks</td>
<td>1105±25</td>
<td>51±11</td>
<td>4.59±0.96</td>
</tr>
<tr>
<td>MWF 25 weeks</td>
<td>974±97</td>
<td>59±16</td>
<td>5.98±1.04</td>
</tr>
<tr>
<td>MWF 50 weeks</td>
<td>1101±131</td>
<td>31±16</td>
<td>2.75±1.06 &quot;&quot;</td>
</tr>
<tr>
<td>MWF 60 weeks</td>
<td>1442±395&quot;&quot;</td>
<td>29±10&quot;&quot;</td>
<td>2.05±0.58 &quot;&quot;</td>
</tr>
</tbody>
</table>

Values are means±SD. * p<0.05 and ** p<0.01 vs. MWF25

All computed parameters were comparable between Wistar and MWF rats at 25 weeks. Among MWF strain, kidney volume significantly increased whereas vascular volume decreased at 60 weeks with respect to 25 weeks (p<0.05, 1-way Anova-Bonferroni). Compared to young animals, significant changes were also observed in the ratio between vascular and kidney volumes at both 50 and 60 weeks (p<0.01, 1-way Anova-Bonferroni).

**Conclusion**

In this study, we showed that MicroCT is a useful tool to analyze morphological and structural changes of the kidney vasculature during the progression of the kidney disease. Specifically, we identified and estimated a simplification of the renal vascular network in MWF rats with advanced nephropathy. How these changes correlate with functional
parameters and can possibly be modulated by therapeutic interventions deserves further investigation.

References: